PEER REVIEWED

Bovine fascioliasis with emphasis on *Fasciola hepatica*

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Abstract

Fasciola hepatica, the common liver fluke, is an economically important parasite of ruminants. Although infections in cattle are generally chronic and sub-clinical, the overall impacts on health and productivity can be significant, including decreased feed efficiency, weight gain, reproductive rates, immunity, immunodiagnostic tests, and responses to vaccinations. Acute infections can occur in cattle, but are more common in sheep. There are no pathognomonic signs of fascioliasis. Fecal examinations using sedimentation or filtration techniques remain the most commonly used diagnostic tools. In the United States, albendazole and a combined clorsulon/ivermectin formulation are the only currently approved products for treatment of liver flukes.

Key words: ruminant fascioliasis, bovine fascioliasis, *Fasciola hepatica,* liver flukes

Résumé

Fasciola hepatica, la douve du foie, est un parasite économiquement important des ruminants. Même si les infections chez les bovins sont généralement chroniques et subcliniques, l'impact dans son ensemble sur la santé et la productivité peut être significatif et se traduit souvent par une diminution de l'efficacité alimentaire, du gain de poids, du taux de reproduction, de l'immunité, des tests immunodiagnostiques et de la réponse à la vaccination. Les infections aiguës s'observent chez les bovins mais sont plus rares que chez les moutons. Il n'y a pas de signes pathognomoniques de la fasciolase. L'examen des fèces avec la technique de sédimentation ou de filtration demeure l'outil diagnostic le plus utilisé. Aux États-Unis, l'albendazole et la formulation combinée clorsulon/ivermectin sont les seuls produits actuellement approuvés pour le traitement des douves du foie.

Introduction

Fascioliasis is an important disease of ruminants with economic losses worldwide estimated at over 3 billion dollars annually.[39] Liver flukes have existed for over 135 million years, with the divergent evolution of *Fasciola hepatica* and *F. gigantica* occurring approximately 19 million years ago.[14] In the continental United States, *Fasciola hepatica* is the most common and economically important fluke infecting domestic large and small ruminants. The related species *F. gigantica,* which is common worldwide, has also been reported in the southeastern United States.[24,32] *Fascioloides magna,* normally a parasite of deer, elk, and moose, also occurs in cattle as an incidental finding at necropsy or slaughter, whereas in sheep it is often fatal.[9,38] Previously reported to infect *Bison bison*, recent research efforts to experimentally infect bison with *Fascioloides magna* have not been successful.[10,38] *Dicrocoelium dendriticum* is a smaller and less pathogenic liver fluke of ruminants that is rarely reported in North America, most recently in western Canada.[6,38]

The geographic range of *F. hepatica* in the US now extends from the gulf coast areas northwestward to the Pacific Northwest.[13,38] Although no formal nation-wide surveys of liver flukes have been published, data provided by cooperating parasitologists[a] have previously documented that *F. hepatica* are found in Florida, Georgia, Alabama, Mississippi, Louisiana, Texas, Arkansas, Oklahoma, Kansas, Nebraska, Colorado, Wyoming, Utah, Nevada, California, Oregon, Washington, Idaho, and Montana. *Fascioloides magna* is also found in many of these areas of the United States including the northwest and the upper Midwest from Minnesota eastward through Wisconsin, Michigan, New York, and other Great Lake states.[38] With extensive transportation of potentially infected cattle between different geographic regions, the presence of the proper snail intermediate hosts and appropriate local environmental conditions may be the only factors limiting further spread of liver flukes. This, in addition to periodic and gradual environmental changes, similar to those that have occurred throughout geologic history, will likely continue to alter the geographic range of liver flukes.

Life Cycle

The life cycle of *F. hepatica* (Figure 1) requires both snail intermediate hosts and the final hosts.[1,38] Adult

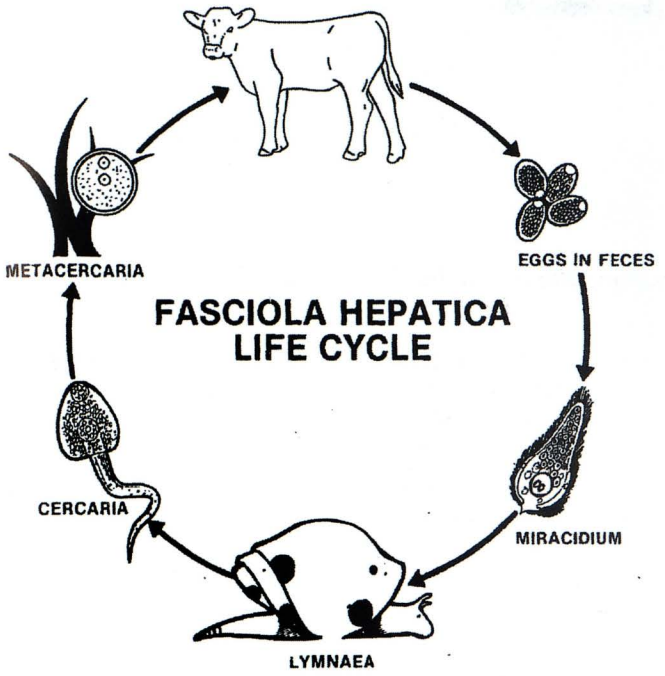

Figure 1. *F. hepatica* life cycle.

flukes (Figure 2) reside in the bile ducts of the liver of the final host (Figure 3). Most trematodes, including liver flukes, are monoecious thus have both male and female reproductive systems in the same individual. Fluke eggs (Figure 4), which have a cap (operculum) on 1 end, are passed in feces. In a moist/aquatic environment, at temperatures from 50°F to 86°F (10°C to 30°C), a ciliated miracidium develops in each egg (Figure 5). When exposed to sunlight and adequate temperature, the operculum opens releasing the free-swimming miracidium which seeks out and penetrates the proper snail intermediate host (Figure 6). These aquatic lymnaeid snails include, but are not limited to, *Galba truncatula* (formerly known as *Lymnaea truncatula*), *L. modicella*, *L. bulimoides*, *Pseudosuccina columnella*, and *Fossaria cubensis*. Within the snail, the sequential developmental stages are the sporocyst, rediae (Figure 7) and the tadpole shaped cercariae. Once they emerge from the snail, the cercariae encyst as metacercariae (Figure 8) on vegetation, inanimate objects, or even on the snails themselves (Figure 6). Metacercariae survive best in aquatic and high humidity environments, but they can survive for weeks on moist hay and can overwinter and remain infective in some areas. Grazing ruminants are infected upon ingesting the metacercariae.

Following ingestion, *F. hepatica* metacercariae excyst in the duodenum. Juvenile flukes penetrate the intestinal wall into the abdominal cavity within 24 hours post-infection. Within 4 to 6 days most juvenile flukes have migrated to and penetrated the liver capsule. The

Figure 2. *F. hepatica* mature fluke.

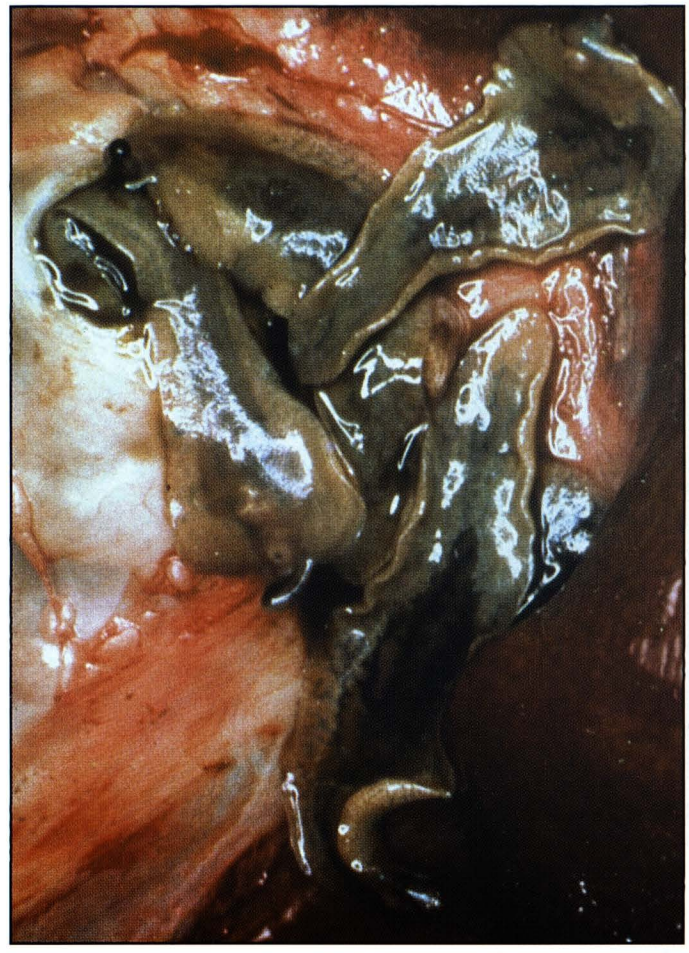

Figure 3. *F. hepatica* mature flukes in situ.

juvenile flukes continue migration through the liver parenchyma, and by approximately 7 weeks post- infection the majority of them have burrowed into the major bile ducts. By 8 weeks post-infection most of them have matured to the large (15 mm by 30 mm) sexually mature egg laying adults. A portion of the population may, however, be delayed or arrested in development. Some

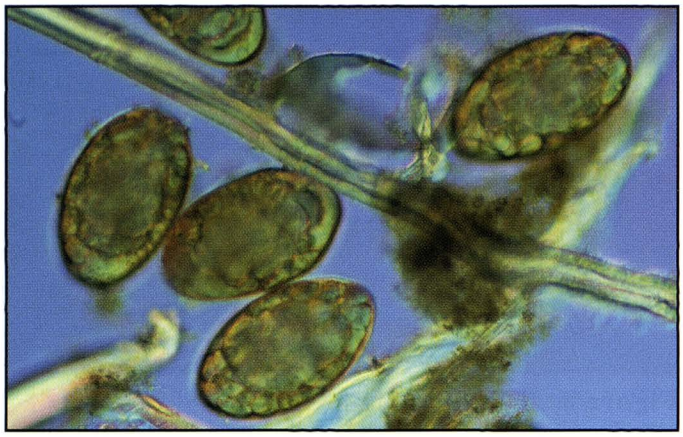

Figure 4. *F. hepatica* eggs.

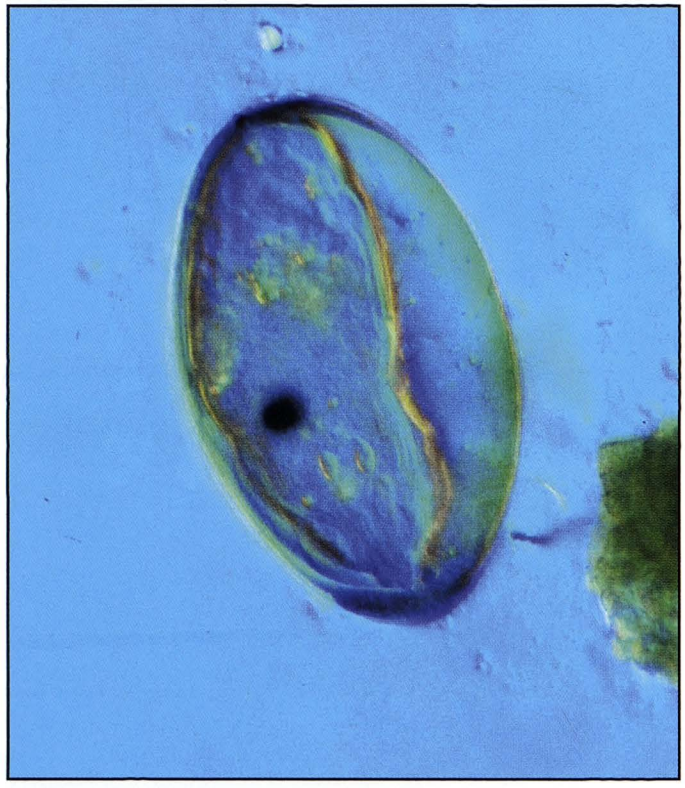

Figure 5. *F. hepatica* miracidium within egg.

immature flukes may migrate to various other organs. The pre-patent period is at minimum 8 weeks and can be as long as 12 weeks.[38]

Pathogenesis and Clinical Findings

Throughout the course of infection flukes cause damage to host tissues. Initial migration tracks on the liver surface are visible by gross examination (Figure 9). The continued migration tracts and fibrosis deep in

Figure 6. *Lymnaea spp* snail (intermediate host of F. hepatica) with F. hepatica metacercariae on the surface.

liver tissue can be severe (Figure 10). Hepatomegaly (Figure 11) as well as gall bladder enlargement is common. Microscopic spines present on the tegument of the flukes as well as excretory-secretory products irritate the bile ducts which become thickened (Figure 12).[20] Inflammatory responses and subsequent calcification of bile ducts during chronic infections results in the classic "pipestem" liver (Figure 13).[38]

Compared to other infectious disease agents and helminth parasites, the size, weight, and surface area of individual liver flukes are significantly greater. This translates into foreign agents with greater potential impacts on the host due to more metabolic products and more tegumental and secretory-excretory antigens which have been shown to be quite complex.[18,21,46] The relatively large amounts of these antigens have numerous adverse effects on host tissues, physiology, metabolism, and the immune system.[3,7,12,14,15,18,19,21,31,45,46] Of particular significance is the ability of *F. hepatica* to

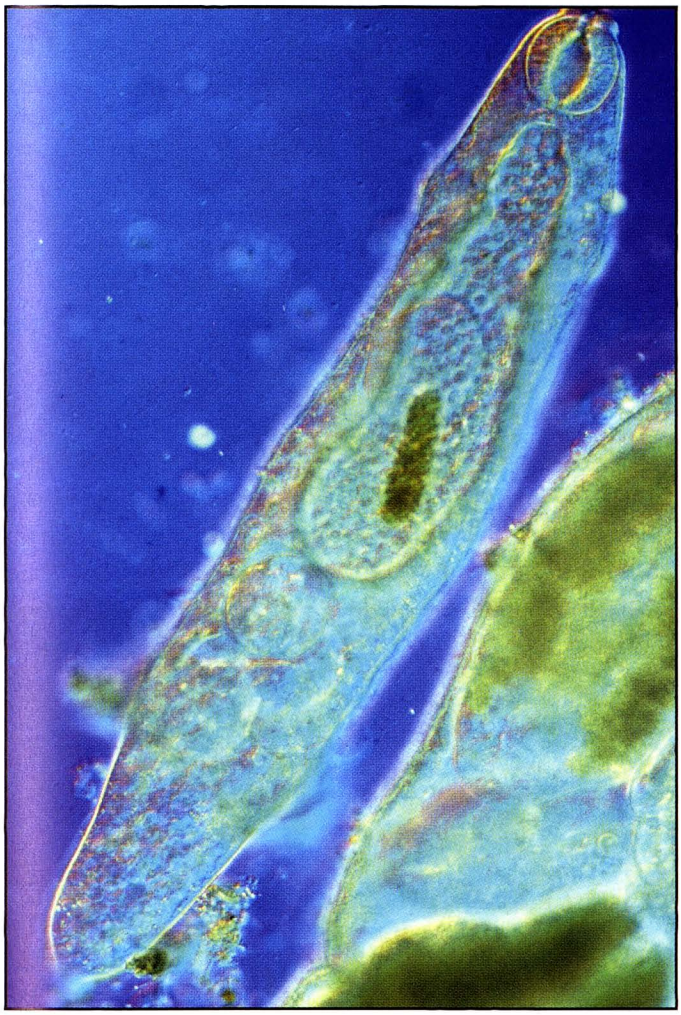

Figure 7. *F. hepatica* rediae.

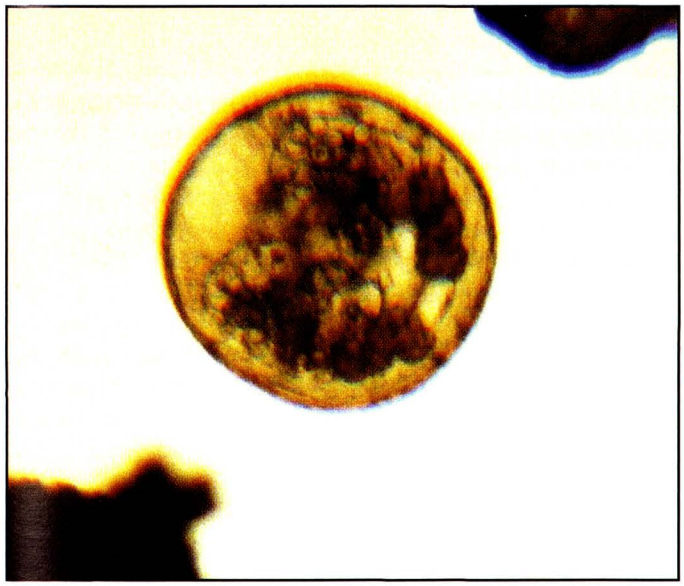

Figure 8. *F. hepatica* metacercariae.

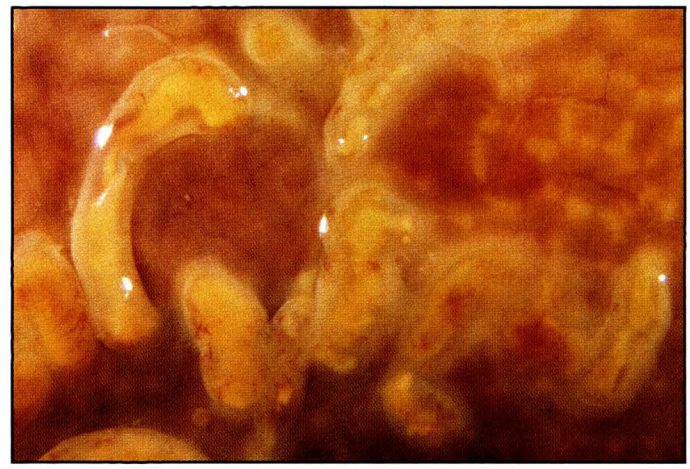

Figure 9. Liver surface with early migration tracts of *F. hepatica*.

Figure 10. Enlarged bile ducts of liver with chronic *F. hepatica* infection.

alter or decrease responses to immunodiagnostic tests, such as bovine tuberculosis.[2,5] Such false-negative diagnostic tests could represent a serious problem, and result in the spread of serious diseases.

Because there is differential susceptibility and host responses of individual animals to *F. hepatica*, all members of a herd may have been exposed to and ingested infective metacercariae, but not all herd cohorts may display the same degree or spectrum of clinical signs of fluke infections.

Although death from liver flukes in cattle is rare, necrotic foci in infected livers can result in anaerobic

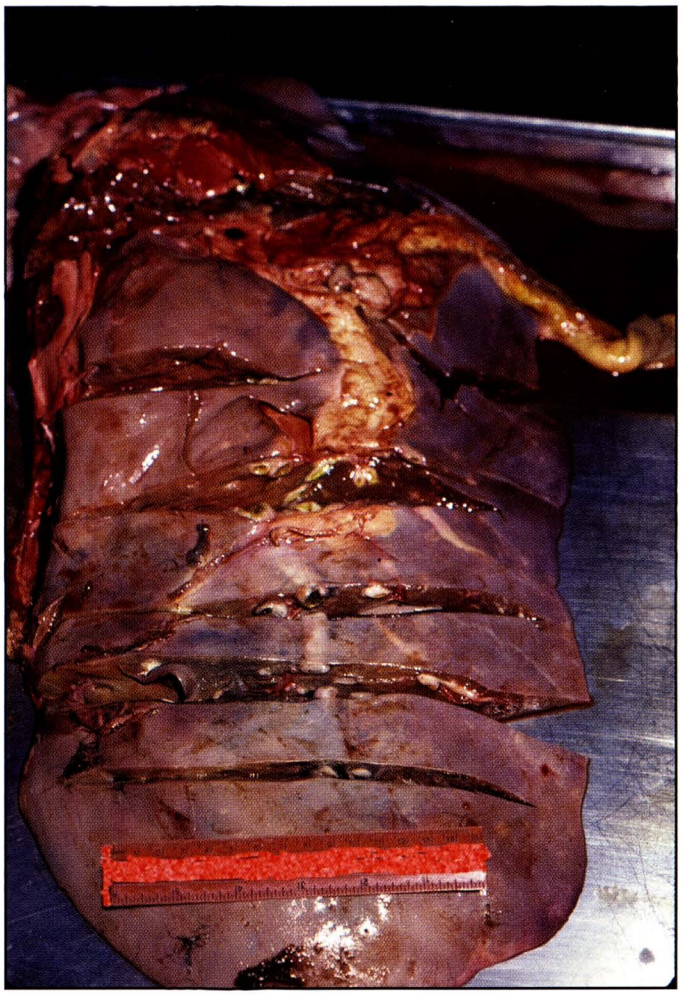

Figure 11. Enlarged fibrotic bovine liver with chronic *F. hepatica* infection.

Figure 12. Cross section of calcified bile duct in chronic *F. hepatica* infection.

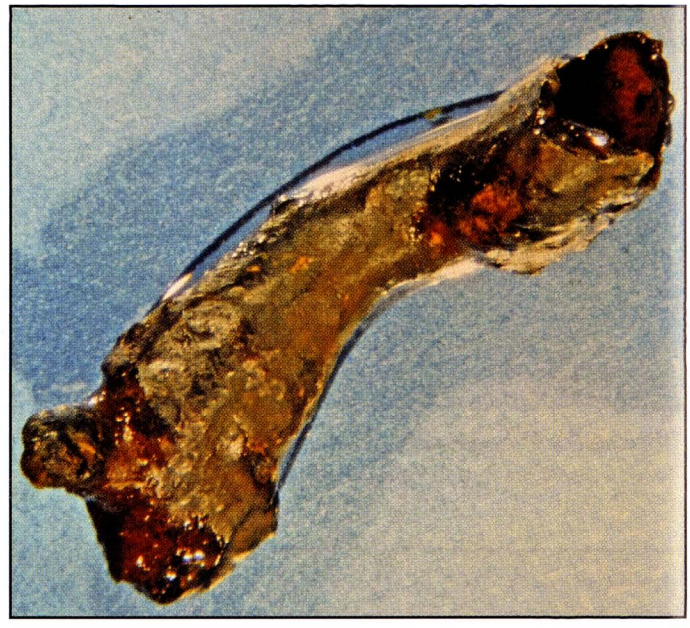

Figure 13. Calcified ("pipe stem") bile duct lining removed from chronic *F. hepatica* infected liver.

conditions favoring proliferation of secondary infections such as *Clostridium novyi* type B and *C. hemolyticum* (*C. novyi* type D).[36,37] Liver fluke infections in sheep are far more serious, causing more extensive liver damage and death.

Fascioloides magna is a large fluke (26 mm by 100 mm) that is less common in cattle. Because they become encapsulated in individual cysts in the bovine liver, these fluke infections are never patent and are generally an isolated finding at necropsy. In small ruminants, however, these flukes do not encapsulate and they migrate throughout the liver, and aberrant systemic migration also causes severe damage that can be fatal.[9,38]

Performance Impact

Liver flukes have a significant economic impact on livestock production. Livers with migration tracts and scar tissue from flukes are condemned at slaughter. The National Beef Quality Audit-2000 reports liver flukes as 1 of the top 10 beef quality issues, with 21.7% of livers condemned for liver flukes.[23] Liver flukes have significant impact on cow-calf herds. Compared to controls, treating cows for liver flukes can result in conception up to 2 weeks earlier as well as higher birth weight and calf weights at weaning.[30] Infected replacement heifers can take significantly longer to reach breeding age.[30] Other effects on production include lower feed efficiency, lower body condition scores, and negative impacts on carcass quality. Combined ivermectin and clorsulon treatment of fluke infected feedlot cattle can increase gains by

over 0.88 lb (400 gm)/day.[16] Decreased milk production in lactating beef cows translates to lower calf weights and even lower weaning weights. Fluke infections in lactating dairy cows significantly lower milk production. Although current products are not approved for use in lactating dairy cows in the US, various studies in the US and other countries have demonstrated milk production losses in untreated cows, depending upon fluke burdens, of 0.45 to 2.3 lb (1 to 5 kg)/day compared to those treated for liver flukes.[4,27,29]

Liver fluke and other parasitic infections have a major impact on host immunity. In order to survive in otherwise immunocompetent hosts, parasites have evolved mechanisms to evade, avoid or suppress the immune system of their host. Most parasites do not live in immunoprivileged sites within the host. As such their survival within a host may depend upon their ability to actively suppress host immune responses. This suppression may be against specific responses directed against the parasite or an overall suppression of host immunity.[49,50,51] Cathepsin L proteases secreted by *F. hepatica* function in tissue invasion and suppression of host immune systems.[28] Liver flukes decrease various T and B lymphocyte functions,[2,45] and decrease the predictive capacity of diagnostic tests such as bovine tuberculosis (BTB) by 27 to 38%.[3,5] Altering macrophage function may explain increased susceptibility of parasitized animals to other infectious agents or decreased responses to vaccines,[8] which could explain some cases previously considered as vaccine failures. Although the negative impact of parasitic infections such as *F. hepatica* is well documented, flukes have been shown to suppress certain autoimmune diseases.[41]

Diagnosis

In endemic areas, liver flukes should be considered in the differential diagnosis of animals showing signs of poor nutrition, weight loss, bottle jaw, diarrhea, low reproductive performance, poor response to vaccines, and increased susceptibility to other diseases.[38,48,49,50,51]

Blood abnormalities in fluke infected animals may include anemia, hypoalbuminemia, hypoproteinemia, eosinophilia, elevated IgE levels, and elevated liver enzymes such as glutamate dehydrogenase (GLDH), gamma-glutamyl transferase (g- GT) and lactate dehydrogenase (LDH), which result from altered or decreased liver function.[38,48] A differential diagnostic problem is that liver fluke endemic areas may also have hepatotoxic plants which, when ingested, can also result in elevated liver enzymes. None of these findings are pathognomonic for liver flukes.

In potentially infected herds, fecal samples from a representative number of herd cohorts should be examined for fluke eggs. The operculum of fluke eggs will open when placed in saturated sugar or salt solutions, and the eggs will sink and not be recovered during normal fecal flotation techniques commonly used for nematode and tapeworm eggs and protozoan cysts. Fecal examinations specific for fluke eggs are necessary. For many years the laboratory diagnostic fecal examination technique to recover liver fluke eggs was an ether-formalin sedimentation technique.[43]

A 2-stage sequential sieving technique can be used to recover fluke eggs found in fecal samples. The normal size of a fluke egg is approximately 63-90 µm by 130-150 µm.[38] A fecal sample is first mixed with water and passed through a 100 mesh (150 µm opening) sieve. The recovered fluid is then poured through a 400 mesh (37.5 µm opening) sieve which will retain the fluke eggs. The debris containing the fluke eggs is backwashed into a small petri dish and stained with new methylene blue.[43] The orange/yellow colored fluke eggs, which do not absorb the stain, will be visible microscopically. Compared to the sedimentation technique above, this sieving procedure is quicker, requires no formalin or ether and is less subject to error. A commercially available sieve kit has simplified finding fluke eggs in feces.[b]

A minimum of 8 weeks post-exposure to infective metacercariae is required to detect fluke eggs in fecal samples.[34] Negative fecal exams during the long prepatent and patent periods do not rule out fluke infection. Several university laboratories developed ELISA diagnostic tests for detecting antibodies in bovine and ovine serum samples.[42,43,44,47] These various laboratory ELISA tests have detected infections as early as 2 to 4 weeks post exposure/infection.[42,44,47] However, these tests may not be currently offered so practitioners should contact diagnostic laboratories for availabilities. Although commercial ELISA based tests (Dot ELISA) were once available, none are currently marketed.

Specific numbers of fluke eggs per gram of feces cannot be used to confirm the absolute level of infection. Even in heavily infected animals, there may be as few as 5 fluke eggs per gram of feces.[12] The ELISA tests are generally not capable of determining fluke burdens. Slaughter records may prove to be 1 of the best sources of determining the infection level and infection potentials at any 1 production facility. Knowing the origin and treatment history of any animals introduced to a facility is an integral part of parasite control and improved herd health.

Treatment and Control

Liver fluke infections are 1 of the top diseases affecting livestock health and production.[26] Livestock producers often regard parasites only in terms of a negative nutritional impact. They may not be aware of the extensive overall effects of these parasites on host

animals and the importance of control programs. They must rely on their veterinarian for knowledge to develop effective total health programs.

The currently available US Food and Drug Administration approved anthelmintics to treat for flukes include combined clorsulon and ivermectin[c] and albendazole.[d] Label instructions should be read for every bottle of any compound administered to animals, including routinely used anthelmintics. Label approvals and directions can be confusing concerning the terms juvenile, immature, and adult flukes. Neither combined clorsulon and ivermectin nor albendazole will effectively kill juvenile flukes (developing flukes not yet in the bile ducts). Once in the bile ducts most of the immature flukes mature to the adult stage. A combined 1% w/v ivermectin plus 10% w/v clorsulon (equivalent to 3.5 mg/kg clorsulon) formulation is effective against mature flukes (8-12 weeks old) and gastrointestinal nematodes, including inhibited *Ostertagia* spp. At the approved label dose, 13.6% albendazole[d] (equivalent to 113.6 mg/ml) is effective against most common nematodes as well as mature liver flukes. Veterinarians should familiarize themselves with label directions before using these products, and pay special attention to residue warnings and precautionary statements. Combined ivermectin/clorsulon[c] should not be used within 49 days of slaughter, in veal calves, or female dairy cattle of breeding age. Label warnings and precautions for the use of albendazole include not using this product in female dairy cattle of breeding age, female cattle during the first 45 days of pregnancy or for 45 days after removal of bulls, and cattle must not be slaughtered within 27 days of the last treatment.

Proper timing of treatments varies with geographic areas. Yearly differences in temperature and moisture patterns in different geographic areas affect the life cycle of flukes and thus transmission times. The survival of fluke eggs as well as all subsequent developmental stages (free swimming miracidiae), the stages within the obligate snail intermediate host (rediae and sporocysts), and the free swimming cercariae require moisture. Metacercariae are susceptible to drying, and thus their survival and infectivity on dry hay also varies with geographic region, weather conditions of specific locations, and practices such as irrigation. Drying of standing water, estivation of infected snails in the summer, and drought are all conditions that reduce infectivity of pastures and vegetation. All of these factors affect the specific time intervals that animals have been exposed to infective metacercariae, which is generally difficult to determine.[17,48,49] These problems led to the development of experimental computer models to attempt to predict transmission times in highly selected areas of the US and limited regions of other countries.[22,23,40,52] However, there are no reports of the use or reliability of these prediction systems throughout the other vast ranges of liver flukes in the US.

Because currently available flukicides are only effective against flukes 8 weeks of age and older, effective treatment should be administered a minimum of 8 weeks after last estimated exposure to metacercariae, such as grazing on potentially infected pastures or being fed fresh hay. For the highest efficacy, this post-exposure time period would be extended to approximately 12 weeks for a combined ivermectin/clorsulon[c] product or albendazole.[d] In more temperate and warmer climates, treatment beginning in mid-summer to early fall will effectively remove flukes acquired in the spring and early summer. In cooler climates, infections acquired throughout the summer require delaying treatment until late fall to early winter.[32] For most all regions, a second treatment should be administered in the spring to remove any residual infections that have matured to stages against which the chosen anthelmintic was initially ineffective. This would also be effective for those animals with late or continued exposure to metacercariae throughout the summer, into the fall, and winter. Any animal with residual infections can be a source of continued pasture contamination. Because flukes can never be totally eliminated in endemic areas, a routine treatment program must be an integral component of good livestock practice and healthcare. As with most helminth parasites, and in this case *F. hepatica* with obligate intermediate hosts and pastures contaminated with infective stages (metacercariae), pasture contamination may represent the greatest percentage of the overall parasite populations. From a parasite's perspective, this is a survival mechanism to insure continued infection of the ruminant final host. From a livestock producer's perspective, it makes fluke control a challenge.

It is important to know the history and origin of animals brought into any livestock operation. Cow/calf operators generally know the infection history for their specific location and may have established appropriate parasite control programs. For stocker, backgrounding, and feedlot operations that bring in cattle from different geographic regions, knowing the infection status may be more difficult. These operations may perform diagnostic tests on representative animals or treat all incoming stock with a broad spectrum combined ivermectin/clorsulon product, administered upon arrival, that is effective against fluke infections acquired 8 weeks or more before entry. For more immature infections an additional treatment may be required depending upon potential fluke and other parasite burdens, such as inhibited *Ostertagia* spp. Because none of these products currently available in the US are approved for lactating dairy cattle, non-confinement dairies in fluke endemic areas must rely on prevention of infections.

There are multiple reports of resistance to flukicides, such as triclabendazole, that are used throughout Europe.[11,34] None of these compounds are approved for or available for use in the US.

Although there are no current vaccines to prevent liver fluke infections, promising research continues with the goal of developing effective vaccines.[7,15,28,39] There are no environmentally appropriate or approved compounds in the US to control snail populations. Treatment of infected animals can reduce pasture contamination. Standing water in areas frequented by cattle that trample vegetation results in a mud habitat that is preferred by the lymnaeid snails. Such conditions are conducive to transmission of liver flukes. Decreasing available snail habitat can lower infection potential by reducing exposure to infective metacercariae.

Conclusions

Liver flukes have a significant impact on health and production of ruminants in large regions of the US. Although there are anthelmintic compounds available to treat for fluke infections, treatment is only a part of a comprehensive control program. Because the fluke life cycle includes snails as obligate intermediate hosts, restricting access to snail infested areas is an important part of the control program. Like many other parasite populations, a significant percentage of the fluke population is in the environment and not in the final host; this is a basic concept of parasitology. Therefore an important goal of the control program is to reduce pasture contamination, thus decreasing the potential of future exposure.

Endnotes

[a]Former USDA, CSRS, W-102 Regional Parasitology Research Program. GL Zimmerman, unpublished data.
[b]Flukefinder®, Flukefinder, Soda Springs, ID
[c]Ivomec Plus®, IvomecF®, Merial Limited, Duluth, GA
[d]Valbazen®, Pfizer Animal Health, Groton CT

Acknowledgement

The author declares no conflict of interest.

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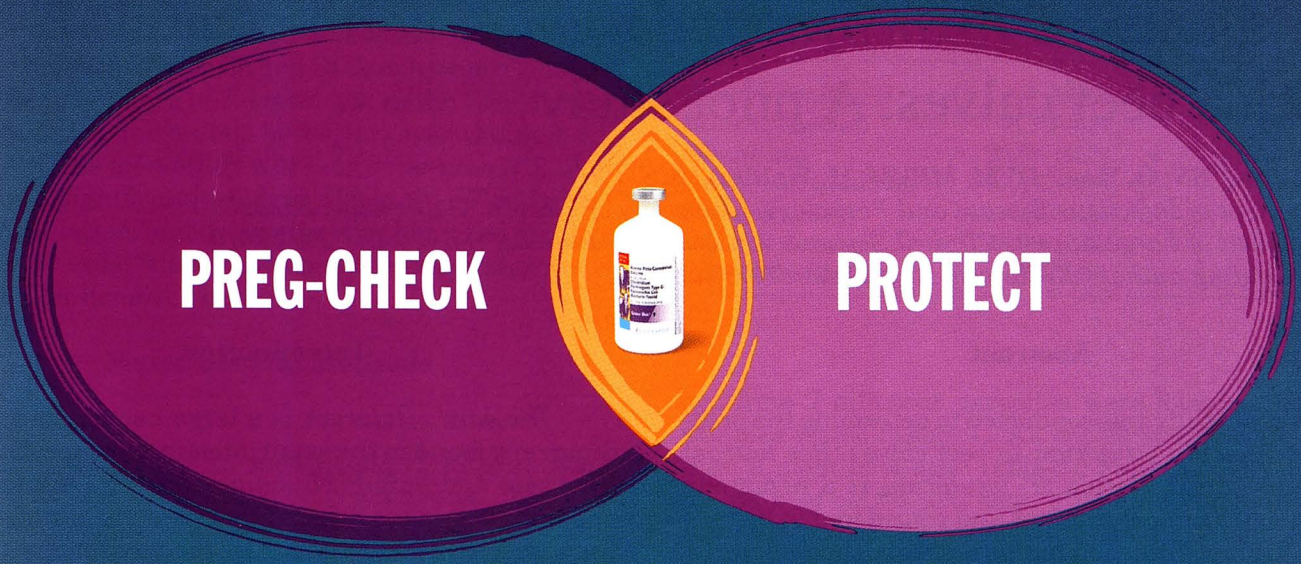

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